

UNCLASSIFIED

AD 409 502

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

63. 4-2

409502

AS AL

FINAL REPORT ON

CONTRACT NO DA 92-557-FEC-34980

INCLUSIVE DATES 15 June 1962 TO 14 June 1963

SUBJECT OF INVESTIGATION

MECHANISMS OF SMOOTH MUSCLE

RELAXATION THROUGH

THE ANODAL CURRENT STIMULATION

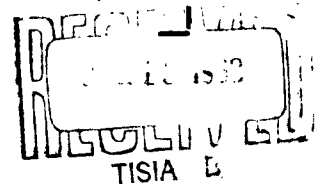
RESPONSIBLE INVESTIGATOR

Dr. Hiroshi Irisawa

Professor of Physiology

School of Medicine

Hiroshima University



U.S. Army Research & Development Group (9852) (Far East)

Office of the Chief of Research and Development

United States Army

APO 343

409502

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Irisawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Irisawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

UNCLASSIFIED

1. Muscles - Stimulation

I. Title: None
II. Irisawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Irisawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Irisawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

UNCLASSIFIED

1. Muscles - Stimulation

I. Title: None
II. Irisawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

UNCLASSIFIED

1. Muscles - Stimulation

I. Title: None
II. Irisawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

UNCLASSIFIED

1. Muscles - Stimulation

I. Title: None
II. Irisawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

that the refractory period of the ureter smooth muscle cell was very long compared to other smooth muscles. The effect of the sodium deficiency on the pattern of action potential has also been studied, and the results indicated that the action potential of the smooth muscle of the ureter can be satisfactorily explained from the basis of sodium hypothesis.

Continuation of the histological works have been made on the fine structure of the invertebrate muscle. The conduction of excitation in the stomatopod heart is definitely different from that of the mammalian smooth muscles, where muscle-to-muscle conduction can be considered. (Author)

UNCLASSIFIED

DESCRIPTORS

Muscles
Stimulation
Instrumentation

UNCLASSIFIED

DESCRIPTORS

Muscles
Stimulation
Instrumentation

that the refractory period of the ureter smooth muscle cell was very long compared to other smooth muscles. The effect of the sodium deficiency on the pattern of action potential has also been studied, and the results indicated that the action potential of the smooth muscle of the ureter can be satisfactorily explained from the basis of sodium hypothesis.

Continuation of the histological works have been made on the fine structure of the invertebrate muscle. The conduction of excitation in the stomatopod heart is definitely different from that of the mammalian smooth muscles, where muscle-to-muscle conduction can be considered. (Author)

UNCLASSIFIED

DESCRIPTORS

Muscles
Stimulation
Instrumentation

UNCLASSIFIED

DESCRIPTORS

Muscles
Stimulation
Instrumentation

UNCLASSIFIED

UNCLASSIFIED

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Iriawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Iriawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

UNCLASSIFIED

1. Muscles - Stimulation
- I. Title: None
II. Iriawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

UNCLASSIFIED

1. Muscles - Stimulation
- I. Title: None
II. Iriawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Iriawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

AD Div 16/1

Hiroshima University, School of Medicine (Japan)
MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH
THE ANODAL CURRENT STIMULATION, by
Hiroshi Iriawa. Final report No. 2, 15 Jun 62
to 14 Jun 63, 17 p. illus, tables, 29 refs.
(Contract DA 92-557-FEC-34980)Unclassified report

Continuation of the studies on the mechanisms
of the smooth muscle relaxation through the
anodal current stimulation has been performed.
In order to conduct the experiment on the mam-
malian smooth muscle, its normal action potential
has been studied. Ureter was selected as the
material because the intracellular action poten-
tial of this tissue has rarely been studied.

Action potential of the ureter smooth muscle
shows a slow plateau phase, which indicates that
a single cell is responsible for the plateau
formation. The effect of repetitive stimula-
tion on this muscle was studied and it was found

UNCLASSIFIED

1. Muscles - Stimulation
- I. Title: None
II. Iriawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

UNCLASSIFIED

1. Muscles - Stimulation
- I. Title: None
II. Iriawa, Hiroshi
III. U.S. Army Research
and Development Gp
(FE), OCRD, DA,
Wash, D. C.
IV. Contract DA 92-557-
FEC-34980

Armed Services
Technical Information
Agency

UNCLASSIFIED

that the refractory period of the ureter smooth muscle cell was very long compared to other smooth muscles. The effect of the sodium deficiency on the pattern of action potential has also been studied, and the results indicated that the action potential of the smooth muscle of the ureter can be satisfactorily explained from the basis of sodium hypothesis.

Continuation of the histological works have been made on the fine structure of the invertebrate muscle. The conduction of excitation in the stomatopod heart is definitely different from that of the mammalian smooth muscles, where muscle-to-muscle conduction can be considered. (Author)

UNCLASSIFIED
DESCRIPTORS

Muscles
Stimulation
Instrumentation

UNCLASSIFIED
DESCRIPTORS

Muscles
Stimulation
Instrumentation

that the refractory period of the ureter smooth muscle cell was very long compared to other smooth muscles. The effect of the sodium deficiency on the pattern of action potential has also been studied, and the results indicated that the action potential of the smooth muscle of the ureter can be satisfactorily explained from the basis of sodium hypothesis.

Continuation of the histological works have been made on the fine structure of the invertebrate muscle. The conduction of excitation in the stomatopod heart is definitely different from that of the mammalian smooth muscles, where muscle-to-muscle conduction can be considered. (Author)

UNCLASSIFIED

UNCLASSIFIED
DESCRIPTORS

Muscles
Stimulation
Instrumentation

UNCLASSIFIED

UNCLASSIFIED
DESCRIPTORS

Muscles
Stimulation
Instrumentation

that the refractory period of the ureter smooth muscle cell was very long compared to other smooth muscles. The effect of the sodium deficiency on the pattern of action potential has also been studied, and the results indicated that the action potential of the smooth muscle of the ureter can be satisfactorily explained from the basis of sodium hypothesis.

Continuation of the histological works have been made on the fine structure of the invertebrate muscle. The conduction of excitation in the stomatopod heart is definitely different from that of the mammalian smooth muscles, where muscle-to-muscle conduction can be considered. (Author)

UNCLASSIFIED

UNCLASSIFIED

D-I-S-T-R-I-B-U-T-I-O-N

The distribution of this report as made by USA R&D Gp (FE)
is as follows:

Army Research Office, 3045 Columbia Pike, Arlington 4, (4)
Virginia. ATTN: Chief Research Programs Office

Army Attache, American Embassy, Tokyo, Japan (1)

U. S. Army Medical R & D Command (4)

ASTIA (10)

Office of Primary Scientific Liaison (1)
Chief, Pharmacology Branch
Directorate of Medical Research
U.S. Army Cal R&D Labs
Edgewood Arsenal, Md

Offices of Scientific Cognizance (4)
Chief, Surgical Research Branch
U.S. Army Med R&D Comd
ATTN: Lt Col Glen
TSG, DA, Wash 25, D. C.

ABSTRACT

Continuation of the studies on the mechanisms of the smooth muscle relaxation through the anodal current stimulation has been performed. In order to conduct the experiment on the mammalian smooth muscle, its normal action potential has been studied. Ureter was selected as the material because the intracellular action potential of this tissue has rarely been studied.

Action potential of the ureter smooth muscle show a slow plateau phase, which indicates that a single cell is responsible for the plateau formation. The effect of repetitive stimulation on this muscle was studied and was found that the refractory period of the ureter smooth muscle cell was very long compared to other smooth muscles. The effect of the sodium deficiency on the pattern of action potential have also been studied, and the results indicated that the action potential of the smooth muscle of the ureter can be satisfactorily explained from the basis of sodium hypothesis.

Continuation of the histological works have been made, on the fine structure of the invertebrate muscle. The conduction of excitation in the stomatopod heart is definitely different from that of the mammalian smooth muscles, where the muscle-to-muscle conduction can be considered.

MECHANISMS OF SMOOTH MUSCLE RELAXATION THROUGH THE ANODAL
CURRENT STIMULATION

———A Survey on the Action Potential of the Ureter
Muscle.

TABLE OF CONTENTS

(1) Introduction	1
(2) Analysis of the problem	3
(3) Outline of the experimental procedure ...	3
(4) Action potential of the ureter musclenormal pattern.....	4
(5) Action potential pattern during the refractory period	5
(6) Effect of the temperature change on the action potential pattern	6
(7) Action potential pattern in sodium deficient Ringer's solution.	7
(8) Anodal current and ureter muscle.	8
(9) Further studies on fine structure of the STOMACH myocardium.	9
(10) Discussion	10
(11) Conclusion	14
Acknowledgement	14
References	15
Appendix	21 illustrations

(1) INTRODUCTION

General review of the problem

Continuous hypertonicity of the smooth muscle has been a hazardous symptom in the patients who complain of their chronic gastrointestinal diseases. The mechanism of the relaxation in smooth muscles have already been studied mainly from the point of mechanical tension, and experiments on their membrane potential are relatively few. In the preceding report, we have studied the relaxation in invertebrate myocardiums through the anodal current pulses, and have found that the anodal current pulse caused an abolition of plateau phase of the action potential in some muscles. While in other muscles such as in the long tubular heart of stomatopod, the anodal current caused the abolition of action potential and the associated inhibition in the development of mechanical tension. The mechanisms of this abolition of plateau is attributed to the anodal block. To apply the anodal current to the ureter smooth muscle, the understanding of the intracellular action potential pattern of this muscle is most essential. The following report deals with the action potential of the ureter smooth muscle with the aid of ultramicro-electrodes.

Review of the previous references

a) Pattern: Bozler (4) injured one part of the ureter and obtained a monophasic potential ranging from 1 to 15 MV and have found that the potential consists of a fast deflection followed by a prolonged negativity. Such prolonged negativity has also been described by Sleater and Butcher (26) in in situ canine ureter, and rat ureter by Prosser et al (25). Greven (11,12) applied a capillary glass electrode having a tip diameter of about 10 microns on ureter and has obtained a similar pattern of action potential from the smooth muscles. Bozler considered that the complex caused by the action potential which is found in guinea pig ureter refers to the individual fiber and is not due to a synchronous activity of the fibers. Greven, on the other hand, considered that the ureter action potential is uniform, and does not show signs of oscillations in its normal condition. The oscillation of the potential in the ureter action potential occurred when the muscle lost the stability of the membrane (11, 12).

In the previous report, we have described a preliminary experiment on the pattern of action potentials from the guinea pig ureter and have found the oscillatory potentials which are superimposed at the initial part of the plateau phase. Bennett, Burnstock, Holman and Walker (2) also reported a brief report apart from us, and found similar action potentials which were

obtained by Bozler (4). We also have come to a similar conclusion that the oscillatory type action potentials are generated from a single muscle cell.

Since the action potential of the ureter muscle has not yet been fully studied with ultramicroelectrodes, it is hoped to study in detail the basic physiology.

b) Refractory period: Bozler obtained conduction velocity of a few centimeters per second and a long refractory period (1.5 to 3 second) without summation of response. Prosser et al obtained a reduced duration of action potential when the ureter was stimulated repetitively. They could not record a reduced amplitude at short intervals between the shocks. But, during the relative refractory period, Ichikawa and Ikeda (18) found a small action potential. Since all these studies were conducted with surface electrode investigation on the intracellular potential recording technique appears to disclose a new approach to this basic problem.

c) Ion effects on the smooth muscle action potentials: It is known that in the ionic theory the presence of sodium ion is a prerequisite in the production of action potential in excitable tissues. Thus the progressive depletion of sodium ion in the extracellular fluid will produce a gradual decrement of action potential height, without appreciable change in the level of the resting potential. This principle has been applied to the giant axon (13, 14), frog skeletal muscle (24) and frog myocardium (5). In the frog myocardium, the substitution of either choline or sucrose in place of sodium chloride resulted in a progressive decrease in height, duration and the rate of rise of action potential.

Holman (16) found in smooth muscle that no change in spike configuration was detected when the concentration of sodium ion was varied between 17 to 150 mM, irrespective of the different substitutes employed. Furthermore, Burnstock and Straub (8) stated that the taenia coli can be excitable for as long several minutes after the total extracellular sodium was removed. Bulbring and Kuriyama (7) found the difference of electrophysiological responses in intestinal smooth muscle when various chemical substances were employed as sodium substitutes. In regard to the various chemical substances Prosser et al (25) found the prolongation of action potential when the tissue is immersed in a solution where 50% NaCl is replaced by choline chloride. Investigation on the intracellular potential recording technique appears to be required in order to evaluate the applicability of sodium hypothesis in the ureter smooth muscle.

(2) ANALYSIS OF THE PROBLEM

The smooth muscles of the mammalian ureter have been studied with surface electrodes as already mentioned in the introduction. The method employed by Bozler (4) was an injury electrode, from which a large number of active cells were responsible for the action potentials. Prosser et al employed the silver-silver chloride electrode for the recording of the ureter action potential. In the following experiments, an intracellular recording technique was used.

(3) OUTLINE OF THE EXPERIMENTAL PROCEDURE

Cat weighing approximately 2 to 2.5 Kg and the guinea pig weighing about 500g were used throughout this experiment. In both species, nembutal was injected intraperitoneally. The ureter was isolated, and made clean by removing the fat and connective tissue under a dissection microscope. The preparation was then placed in warm Ringer-Kreb's solution controlled at 36°C electronically by passing current through the thermojoule element. Ringer's solution was continuously oxygenated. Composition of the normal Ringer-Krebs solution was as follows: NaCl 0.035g, CaCl_2 0.028g, MgCl_2 0.029 g, NaH_2PO_4 0.0165g, NaHCO_3 0.068g in 100 ml of water. Dextrose (0.14g/100ml) was added shortly before the experiment according to the description by Bozler (4). When the amount of NaCl was reduced, sucrose, choline chloride or tris chloride (Tris hydroxymethyl aminomethane) was used to adjust the tonicity of the solution. In the case where tris was used as a substitute of NaCl, pH of the solution was adjusted with HCl instead of NaH_2PO_4 and NaHCO_3 . The remaining components of the solution were unchanged unless otherwise indicated. According to Goodford and Hermansen (10) over 95% of the muscle sodium is exchanged within 5 minutes in Na^{24} solution. Therefore, the muscle used in this experiment was immersed in the solution examined for at least 5 minutes before the recordings were made. Membrane potential was measured by ultramicroelectrodes filled with 3M KCl and having a resistance ranging from 20 to 100 M Ω (23), and the Woodbury and Brady's fluctuating suspending method was used (29). A high input impedance, negative capacity amplifier (Nihon Koden Kogyo MZ-3A) and a cathode ray oscilloscope (Nihon-koden Kogyo VC-6, AVH) were employed. Displayed phenomena were photographed with a Grass type Kymograph camera, and at the same time an ink writing recorder was used in order to monitor the resting potential continuously. Stimulation electrodes consist of two wick electrodes, one of which was placed on the ureter and the other immersed in the bathing fluid. The distance between the stimulating and the recording electrodes was varied from 1 mm to 15 mm. The rectangular current pulses of varied duration and

intensity were applied through electrodes.

(4) ACTION POTENTIAL OF THE URETER MUSCLE----NORMAL PATTERN

a) Cat: The resting potential ranged from 25 MV to 63 MV and the height of the action potential from 17 MV to 69 MV. The mean values were 45 MV and 32 MV, respectively. Among 193 penetrations, there were only 4 cases where the reversal potential was found ranging from 2 to 9 MV (mean 5.6 MV). The values of both the resting and the action potentials were widely distributed and the overshoots of the potentials were rarely found. A typical pattern of action potentials were widely distributed and the overshoots of the potentials were rarely found. A typical pattern of action potential is a spike potential followed by a slow repolarization phase as shown in Figure 1. There are no oscillations on the plateau phase such as found in the normal action potential pattern of guinea pig ureter (21). However, in most instances there appears a hump on the repolarization phase. The maximal rising rate of action potential is about 0.5-0.6V/sec, which is much slower than that of other smooth muscles or myocardium. However, the rate of depolarization is always more rapid than the repolarization rate (about 0.2 V/sec) like those found in the myocardium. This fact differs from that of the intestinal smooth muscle in which the rising rate of action potential is slower than its falling rate.

b) Guinea pig: The resting potential ranged from 37 to 54mV and the height of action potential from 26 to 54mV. The mean values were 49 mV and 38mV respectively. The pattern of action potential which was recorded from guinea pig differed from that of cat in that they showed a longer plateau phase and several superimposed oscillatory potentials. The rising phase of the action potential contains very slow potential changes. Such slow potentials are considered to be either junction potential or electrotonic spread of the activity of the adjacent cells. The individual spike component of the superimposed action potential always differed in height, duration and in the rate of rise of potential. There were two types of spike potentials. One type consisted of a gradual development of small spike potentials reaching its maximum height in the second or the third spikes, followed by a gradual decrease in spike height. The other type was made up of a large spike potential produced at first, followed by a gradual decrease in spike height. In both cases a plateau of about 1 second in duration was observed (Figure 2)

(5) ACTION POTENTIAL PATTERN DURING THE REFRACTORY PERIOD

a) Cat: The recovery processes of the action potential in the ureter muscle of cat was examined. The action potential was elicited with the first shock and the second shock was applied at various time intervals, as is shown in the 5 tracings of figure 3. No response was found when the second stimulus was applied at the early repolarization phase (Fig. 3-1), while very small deflection appeared when it was applied in the latter part of the repolarization phase (Fig. 3-2). When the second stimulation fell at still a later phase, an action potential of a slower rising phase and of a decreased height was recorded (Fig. 3-3 and -4). In the fifth tracing of this figure (Fig. 3-5) where the second stimulus was applied 1.5 seconds after the first stimulus, it is shown that the action potential has almost recovered to its original height, but the rising phase has not yet recovered. Thus, the refractory period of the ureter muscle was found to be more than 1.5 seconds, in this special instance.

As shown in Fig. 4, with the repetitive stimulations, the first action potential showed a steep rise in all cases, but after the second, the rate of rise of potential decreased. When the frequency of stimulation increased, there occurred a small slow potential changes. It was observed through the binocular microscope that this small potential change was not accompanied by any conducted contraction. In order to observe the changes in the rising rate in detail, the successive action potentials which were elicited in response to repetitive stimulations were superimposed (Fig. 5). These illustrations indicate that the rate of rise of potential became slower and slower when the repeated stimulations were made. The slow rate of rise appears to be one of the characteristics of the potentials which developed during the refractory periods. It is also suggested as in C of this figure that the slow potential can summate to develop conducted action potential.

When the potentials were recorded in the ureter at three different places away from the stimulating electrode, the time interval between the shock artefact and the beginning of the action potential differed from each other. It was 0.2 second when the recording was made 3 mm away from the stimulating electrode, 0.7 sec at 1 cm and 1.4 second at 2 cm. thus, it can be said that the time interval is approximately proportional to the distance between the stimulating and the recording electrodes.

b) Guinea pig: The action potentials of guinea pig ureter showed similar trends in response to the repetitive stimuli, except that they showed a longer plateau phase and superimposed several

oscillatory potentials. Figure 6A gives typical examples where each tracing was taken from the continuous records. In tracing 1, the action potential developed 0.3 seconds after the stimulus artefact and 8 distinct oscillations were superimposed on the plateau phase. The second stimulus was given 3.6 second after the stimulus artefact and 8 distinct oscillations were superimposed on the plateau phase. The second stimulus was given 3.6 seconds after the completion of the repolarization of the first action potential (tracing 2). The third stimulus was applied 1.6 seconds later (tracing 3). It can be noticed that as time decreased the number of oscillatory potentials gradually decreased and the duration of action potentials shortened. In Figure 6B the superimposed tracing of these three action potentials is shown in inset. The changes in the rate of depolarization, in the duration of action potential and the conduction velocity are graphically illustrated in this figure, where the inset numerals are indicated as the absolute values in each measurement.

When the smooth muscle of the guinea pig ureter was asphyxiated, the plateau phase disappeared and the spike potential which is similar to the action potential of the taenia coli could be recorded. This potential differs from the normal ureter action potential of guinea pig in a faster rate of spontaneous firing, a faster repolarization phase and an absence of plateau phase. When the stimulus was applied shortly after the spontaneous spike potential, only a single spike appeared (Fig. 7). It can also be seen that the spike potential is followed by a slow after potential. If the stimulation was applied at a slightly later phase, triple spike potentials appeared. Likewise, when the stimulus was applied later in the recovery phase, one could record an increased number of spike potentials. Thus, it was found that even under these conditions the ureter muscle showed repetitive responses to a single external stimulation.

(6) EFFECT OF THE TEMPERATURE CHANGE ON THE ACTION POTENTIAL PATTERN

a) Cat: The temperature of the bathing fluid also influences the rising rate of the action potential. Figure 8 illustrates the effects of temperature on the rising rate and the duration of action potentials in ureter muscle of cat. It can be seen that the lower the temperature, the slower the rate of rise of potential. The fact that the duration of action potential is directly related to the temperature of the bathing fluid is illustrated in this figure. The conduction velocity also decreased in the lower temperature.

b) Guinea pig: Similar experiments have been performed in the ureter muscles of guinea pig (Fig. 9). The action potentials of the ureter muscle in three different bathing temperatures are illustrated. In 35°C the rise time was short, and the interval of two successive spikes was about 50 m sec. In 30°C , the rise time was longer than that in 35°C , and the interval between the two spikes was about 71 m sec. This interval was further decreased to about 95 m sec in 27°C . The decrease in conduction velocity was invariably observed. Thus the effects of temperature on both the cat and the guinea pig ureter were approximately the same from the point of rate of rise of potential and conduction velocity.

(7) ACTION POTENTIAL PATTERN IN SODIUM DEFICIENT RINGER'S SOLUTION

a) Cat: Figures 10 - 12 show the results obtained by the effect of sodium replacement with sucrose, choline chloride and Tris chloride in cat ureter. Fig. 10 illustrates the results where sucrose was used as a substitute of sodium chloride. As can be seen in this figure, the rate of rise of action Potential gradually reduced from 1 to 4 where the extracellular Na was stepwise reduced from normal contour to $1/4$ of a sodium solution. The duration of action potential was prolonged in the lower sodium concentration and also the distance of a conduction of excitation became very limited, approximately a few mm from the stimulating electrode. Also the threshold of the muscle to the stimulation increased. When the ureter was immersed in sodium lack fluid, neither contraction NOR action potential could be observed.

Figure 11 illustrates a similar experiment with choline chloride. The results obtained by substituting (Na) with choline resembled that of sucrose: the rate of rise of potential was decreased, duration of action potential prolonged and the conduction velocity decreased progressively as the extracellular sodium concentration was reduced. A very different trend was found when tris chloride was used as a substitute of NaCl (Fig. 12). The duration of action potential shortened, instead of being prolonged. The rising rate and the conduction velocity showed a reduction which is consistent with the results of sucrose and choline substitution. In the sodium free solution in which all sodium chloride was replaced by tris chloride, the conduction velocity became still slower and only a small potential change was recorded as shown in Fig. 12 - 5. Even when the recording electrode was placed closer to the stimulating electrode, no action potential could be obtained except for a small slow response (Fig 12-6). The response disappeared. About

10 minutes later.

Regards to the resting potential, there were no significant differences statistically among the values in normal and in various kinds of sodium substitutes.

In Figure 13, 14 and 15, the height of action potential, the rising rate and the duration of action potential in three kinds of bathing fluid were plotted against the concentration of extracellular sodium chloride. Each plot is the average value of the action potential height, rising rate and the duration taken from 15 to 30 records. It can be seen that there are slight but definitely recognizable decrease in the height of action potential, when the sodium is reduced. The rate of rise of action potential appears to be directly proportional to the extracellular sodium concentration. Regards to the duration of action potential a significant difference as observed between the Tris chloride substitution and the other two substitutes. In other words, both in sucrose and in choline the duration of action potential prolonged while in Tris chloride, the reverse effect resulted. This indicates that the effect of sodium deficiency on the duration of action potential is caused by the sodium substitutes rather than the reduction of sodium.

b) Guinea pig: Effects of sodium reduction on the action potential pattern of guinea pig were also studied. Figure 16 illustrates the pattern of action potential when the muscle is soaked in the reduced Na solution and replaced by sucrose. The pattern of action potential changed into an irregular form soon after 50 % of sodium in Ringer was replaced by sucrose. The decrease in frequency of spikes, in rate of rise of potential, in conduction velocity were invariable observed. Similar trends were observed when the choline was used instead of sucrose. A remarkable decrease in the frequency of spike formation was definitely observed (Fig. 17). The same was found when Tris chloride was used. The reduction in the rate of rise of potentials and humps, which were noticed prior to the formation of spike potential, were observed (Fig. 18).

(8) ANODAL CURRENT AND URETER MUSCLE

As stated above, in stomatopod, the anodal current caused an anodal block. In oyster myocardium, an abolition of plateau phase has been obtained, and the abolition of action potential is found to be correlated with the relaxation of muscle tension. In ureter muscle, we have not been able to elicit the relaxation by applying anodal current. In one experiment, the cathodal current of a varied intensity and a constant duration were applied on the ureter which had ceased spontaneous

firings. The onset of the current was gradually decreased and the number of spikes elicited increased. The increased spikes soon accommodated as can be seen in the fifth and the last recordings of Fig. 19-1. The application of the anodal current is illustrated in Fig. 19-2. When the current is increased gradually, the spike potential is elicited slightly earlier than the previous one. Still greater increase in the current strength results in an earlier initiation of the spike potential and furthermore the spontaneous response is obtained. This behaviour on the anodal current to this muscle can not be observed when the action potential of the ureter showed a plateau. On the contrary, this was observed when the action potential changed its pattern into a spike type. Sleator and Butcher (26) applied galvanic polarization, and have found when the anode is placed intraureteral, the conduction velocity of the ureter is reduced. They, however, did not observe the relaxation. BULbring (6) observed the relaxation in taenia coli when she applied the anodal current polarization. Our failure on reproducing Dr. BULbring's results appears to have been due to the difference in the method of applying the current.

(9) FURTHER STUDIES ON THE FINE STRUCTURE OF THE STOMATOPOD MYOCARDIUM

In the previous report (19, 22), we have already stated the fine structure of the stomatopod. During the past year, our work was concentrated on the myofibril structures and the sarcoplasmic reticuli. We have observed further fine structures and the sarcoplasmic reticuli. The fine structure of stomatopod heart muscle provides evidence of the existence of two distinct components of sarcoplasmic reticulum. A sarcolemmal invagination was extended deep into the muscle cells, and was transversed at the level of Z-lines of the myofibrils. The other endoplasmic reticulum system can be seen around the myofibrils. The structure of the latter tubular system, in other words, the sarcoplasmic reticulum exhibited a similar structure as that found by Bennett and Porter (1). Figure 20 illustrates the deep invagination of the sarcolemmal membrane, and Figure 21 illustrates one of the typical transverse sections of the mantis shrimp heart. It can be seen that the double filament array (thick filament in center and thin filaments around the orbit) are also noted in this muscle (17). Although the muscle protein of this species is not actomyosin system, the pattern of arrangements in myofilaments are very much like that of the vertebrate myofibril. It also can be seen that there is a rich supply of endoplasmic reticulum outside the group of myofibrils. Although we could not confirm the triad structure

a large tube was found, having a basement membrane which indicates that the tube is directly connected to the outside of the cell. Such infolding of the plasma membrane into the cell is already found in the myocardium of human, dog and rabbit. Thick sarcoplasmic granules were observed outside the myofibril and since they were PAS positive granules, they are considered to be glycogen granules. The mitochondria are present around the muscle fibers. When the longitudinal section of the myocardium is observed, a regular Z-line structure can be seen. No intercalated disc such as seen in vertebrate myocardium was observed. Two adjacent cells were separated considerably, and therefore the muscle cell was not considered as a syncytium. In some of the smooth muscles the distance between the two adjacent cells were connected with a short bridge (3). In vertebrate myocardium, the adjacent cells were tightly connected with desmosome structure and intercalated disc, allowing the current to pass through easily. In the myocardium of the stomatopod, the muscle cells were separated with wide extracellular spaces, but nevertheless the long heart tube was contracting synchronously. This suggests that the coordination of the contraction must depend on the nervous control, and is in good correlation with the physiological observations (20). It is also suggested from the physiological study that the multiterminal junction is present in this heart. The electron micrograph of these two structures are now being studied.

DISCUSSION

In rat ureter, Bennett et al (12) found that the resting potential is about 60 mV and that the action potential may overshoot about 10 - 15 mV. However, in cat ureter, during over 190 penetrations, there appeared only four instances where the action potential showed an overshoot, and the amplitudes of action potentials were comparatively smaller than those in rat ureter reported by Bennett et al. Some of the electrodes might have not been penetrated into the cell, as was suggested by Gillespie (9), or the electrodes may have injured the cells. Although the electrodes employed are of a high resistance before and after the penetration and the resting potential was reasonably stable, many of the data obtained from this experiment showed a low resting potential and a small action potential. Thus, it was very difficult to draw a line between the intracellular and extracellular recordings, objectively. For this reason, the authors did not discard the data which showed low resting and small action potentials in both the control and sodium lack solution.

Bozler (4) recorded extra cellularly a monophasic action

potential in ureter and described that the pattern of action potentials are different from species to species. Greven (11, 12) also recorded a monophasic action potential in guinea pig ureter with an extracellular glass microelectrode. Action potentials which were recorded here with microelectrodes confirmed these authors' results and have shown that delayed action potential is generated from a single smooth muscle cell. This is also in good agreement with Bennett et al (2), and it is suggested that the ureter muscle behaves somewhat like a physiological syncytium. All or none response to the electrical stimulation is known to occur in ureter muscle through the studies of Bozler (4) and Prosser et al (25). From the intracellular recordings of the present experiment, the action potential which showed a slow conduction velocity, a slow rising phase and a short duration was invariably obtained when the ureter smooth muscle was stimulated repetitively. These responses to the repetitive stimulations of the ureter muscle resembled that of the vertebrate myocardium which was discussed by Ficker and Woodbury (5). The slow-small potential which was not observed in the myocardium by high frequency stimulations (2/sec) was obtained in the ureter muscle. This abortive response was also observed by Prosser et al (25) through their extracellular recordings, and appears to be one of the differences between the ureter and the myocardiums.

Another distinct feature of the ureter muscle as compared to the myocardium is its long lasting refractory period. The myocardium is known to recover from its inactivation processes as soon as the membrane repolarizes. However, in the ureter muscle the absolute refractory periods lasted one to three seconds after the membrane potential was completely repolarized. This prolonged state of inactivation processes seems to depend on the duration of the preceding action potential. When the duration of action potential is short, the ureter muscle can respond to the repeated stimulations as fast as once in two seconds.

Eleator and Butcher (26) found in in situ dog ureter that, during the post peristaltic interval the speed with which the second wave travels is less than that of the first. Their findings coincide with the present observation in which an intracellular recording was employed. In their results there was no measurable change in the rate of depolarization or in peak height. However, in the present study a remarkable reduction in rate of rise of potential and a considerable reduction in the peak height of the action potential were recognized. This may be due to the difference in the method between this experiment and the previous one.

The superimposability of the repolarization phase during the repetitive stimulations was also observed in the urter muscles as in the myocardium. In the myocardium, the repolarization was

superimposable when the action potentials were shifted so that the most rapidly falling phase would coincide. While, in the ureter of the guinea pig the superimposability of the repolarization phase could be seen when the stimulus artefacts were superimposed. This indicates that the duration between stimulus and the completion of the repolarization phase is approximately the same regardless of the duration of the action potential.

Bennett et al (2) found a large spike potential when a very high Ca^{++} concentration was applied in guinea pig ureter. The spike potentials which were observed in the present experiment appear to be different in nature, since the magnitude of the spikes in this experiment is much smaller than that observed by Bennett et al. These facts suggest that the action potential of the ureter muscle can change their pattern depending on the external physiological conditions.

The height of the action potential in the myocardium was unchanged until the temperature decreased below 20°C (27). In comparison to the myocardium, the action potential of the ureter appears to be more sensitive to the change of temperature of the bathing fluid. In the ureter muscle, the peak height of the action potential was reduced and both the conduction velocity and the rate of depolarization remarkably decreased in lower temperatures. The resting potential was lowered when the temperature was reduced to 20°C . In guinea pig ureter, the excitation fails to occur below 25°C , while in cat ureter the muscle can respond in a still lower temperature, suggesting the sensitivity of ureter tissue to temperature differs from species to species. Prolongation of action potential also occurred in myocardium when it is cooled, the similar trends were observed in ureter muscle. It may be reasonably stated that the results obtained in the present experiment can be explained from the basis of sodium theory revised by Woodbury (28).

The applicability of the sodium theory to the smooth muscle of the ureter is also demonstrated in the above experiments. The fact that the ureter muscle cell can develop action potential in a very low extracellular sodium concentration coincides with the findings in the intestinal smooth muscle (8, 15). But in this experiment the rising rate and the height of the action potential of the ureter muscle progressively decreased when the extracellular sodium was reduced. As far as the three kinds of sodium substitutes, sucrose, choline chloride and Tris chloride are concerned, the rising rate and the height of action potential were of similar trends, that is, there are no recognizable differences among sodium substitutes, suggesting that the extracellular sodium concentration affects primarily upon those two factors. However, in regard to the duration of action potential both

choline and sucrose showed an entirely different trend from that of Tris chloride. In other words, the action potentials of ureter muscle prolonged in choline or sucrose solution, but showed a shortening in Tris chloride solution. This in turn suggests that the plateau phase of action potential is not mainly affected by the extracellular sodium concentration but by the sodium substitutes.

Hoffman and Crane (15) summarized the present knowledge about the effects of the sodium substitution on mammalian myocardium. It seems reasonable to conclude from their table that many of the preparations showed a shortening of action potential rather than a prolongation in sodium deprived solution. In cat ureter smooth muscle, only the result from Tris chloride solution agrees with the previous results in myocardium.

The prolongation of plateau seen in choline chloride solution confirmed the previous extracellular findings of Prosser et al (25), and presented the concept that the prolongation is not due to the enhancement of the synchronous activities of a group of muscle cells, but due to the phenomenon of a single muscle cell membrane. As atropin also prolongs the action potential plateau in ureter (25), the prolongation appears not to be due to the nervous origin, but is likely due to the direct action on muscle cells.

It was found in the present experiment that the ureter smooth muscle can not develop action potentials in a sodium lack solution, even though it contains the same quantity of other ions such as K^+ , Ca^{++} and Mg^{++} as in normal Ringer-Krebs solution. This indicates that sodium plays the most important part in developing the action potential of the ureter smooth muscle. In ureter muscle, the action potential ceases to develop shortly after the immersion in Ca^{++} free Ringer-Krebs solution (unpublished data). This indicates that Ca ion also plays an important role in the production of action potential. Further studies on the effect of Ca ion on the ureter muscle are under progress. It is our opinion that the basic ion contributing to the normal action potential is sodium ion.

Bergman (3) studied the fine structure of the rat ureter muscle. He observed that the diameter of the smooth muscle is 7 microns at its greatest width, and length about 500 microns. The muscle having 75 to 100 μ myofilaments is covered with 250 μ double membrane. Intracellular spaces are filled with dense bundles of collagen connective tissues and an extensive capillary network. Each muscle cell has several intercellular bridges, thereby joining it with adjacent cells. The intercellular structure is 2 to 3 microns in length and 0.3 - 0.5 microns in diameter. In our preliminary experiment in guinea pig ureter, the cell bridges have not been observed, instead the two adjacent cells are connected with a very short distance between them.

This fact suggests that the fine structure of the muscle arrangement is different from species to species. Further study on the observation in their muscles appears to be necessary.

(11) CONCLUSION

Action potential of the smooth muscle of the ureter has been studied with the ultramicroelectrodes. Intracellular action potential of the ureter shows a plateau phase which is similar to that found in the myocardium. The plateau phase can be shortened through repetitive stimulations. The superimposability of the action potential was observed. The action potential plateau can be shortened in sodium deficient solution replaced by Tris chloride solution. The plateau phase of the ureter action potential thus found to show similar characteristics to that of the vertebrate myocardium. Anodal current pulse did not cause the abolition of action potential such as observed in the invertebrate myocardium. By devising the method of current application to this muscle, we hope to elicit the relaxation due to anodal polarization in the future. The histological studies on the invertebrate muscle confirmed the two types of tubular systems, which may be responsible to the excitation and contraction coupling in this muscle. Such structures have been found in many vertebrate muscles, but in the smooth muscles no such structure have been demonstrated. Therefore, the conduction of excitation in smooth muscles appears to have a different system and awaits further study.

ACKNOWLEDGEMENT

I am deeply appreciate to Dr. G. B. Darling: Director of A. B. C. C. Hiroshima for his goodwill of helping us during the interruption of our fine structure study.

REFERENCES

- (1) Bennett, H.S. and Porter, K.R. 1953 An electron microscope study of the sectional breast muscle of the domestic fowl. Am.J.Anatomy 93, 61
- (2) Bennett, M.R., Burnstock, M.E. Holman and J.W.Walker 1962 The effect of Ca^{2+} on plateau-type action potentials in smooth muscle. J.Physiol. 161, 47-48 P
- (3) Bergman, R.A. 1958 Intercellular bridges in ureter smooth muscle. Bull.John Hopkins Hospital 102, 195-202
- (4) Bozler, E. 1942 The action potentials accompanying conducted responses in visceral smooth muscles. Am.J.Physiol. 136, 553-560
- (5) Brady, A.J. and J.W.Woodbury 1960 The sodium-potassium hypothesis as the basis of electrical activity in frog ventricles. J.Physiol. 154, 385-407
- (6) Bulbring, E. 1955 Correlation between membrane potential, spike discharge and tension in smooth muscle. J.Physiol. 128, 200-221
- (7) Bulbring, E., H.Kuriyama and Betty Twarog 1962 Influence of sodium and calcium on spontaneous spike generation in smooth muscle. J.Physiol. 161, 48-49P
- (8) Burnstock, G. and R.W.Straub 1958 A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. J.Physiol. 140, 156-167
- (9) Gillespie, J.S. 1962 The electrical and mechanical responses of intestinal smooth muscle cells to stimulation of their extrinsic parasympathetic nerves. J.Physiol. 162, 76-92
- (10) Goodford, P.J. and K.Hermansen 1961 Sodium and potassium movements in the unstriated muscle of the guinea-pig taenia coli. J.Physiol. 158, 426-448
- (11) Greven, K. 1953 ⁴Über ruhe und Aktionspotentiale der glatten Muskulatur nach Untersuchungen mit Glaskapillarelektroden. Zschr. Biol. 106, 1-15.

- (12) Greven, K. 1956 Über örtliche und zeitliche Variationen im Erregungsablauf am Meerschweinchen-Ureter. Zschr.Biol. 108, 431-438
- (13) Hodgkin, A.L. 1951 The ionic basis of electrical activity in nerve and muscle. Biol. Rev. 26, 339-409
- (14) Hodgkin, A.L. and B.Katz 1949 The effect of sodium ion on the electrical activity of the giant axon of the squid. J.Physiol. 108, 37-77
- (15) Hoffman, B.F. and P.F. Cranefield 1960 Electrophysiology of the heart. New York McGraw-Hill, p276.
- (16) Holman, M.E. 1957 The effect of changes in sodium chloride concentration on the smooth muscle of the Guinea pig Taenia coli. J.Physiol. 136, 569-584
- (17) Huxley, H.E. and J.Hanson 1960 The molecular basis of contraction in cross- striated muscles. MUSCLE- structure and function of muscle 1 : 183-227
- (18) Ichikawa, S. and O.Ikeda 1960 Recovery curve and conduction of action potentials in the ureter of guinea pig. J.J. Physiol. 10, 1-12
- (19) Irisawa, H. 1962 Final report on U.S.Army Research & Development Group (Far East)
- (20) Irisawa, H., A.Irisawa, T.Matsubayashi and M.Kobayashi 1962 The nervous control of the intracellular action potential of the Squilla heart. J.Cell and Comp. Physiol. 59, 55-60
- (21) Irisawa, H. and M.Kobayashi 1962 Intracellular action potentials of the Guinea pig ureter. Proc.Japan Academy. 38, 171-175
- (22) Irisawa, H., A.Irisawa and M.Kobayashi 1962 Relaxation of invertebrate myocardium through the anodal current pulse. 22nd Int.Congr. Physiol. Sci. (Leiden)
- (23) Ling, G. and R.W. Gerard 1949 The normal membrane potential of frog sartorius fibers. J.Cell and Comparative Physiol. 34, 383-396
- (24) Nustuk, W.L. and A.L.Hodgkin 1950 The electrical activity of single muscle fibers. J.Cell and Comp. Physiol. 35, 39-73

- (25) Prosser, C.L. C.E. Smith and C.E. Melton 1955
Conduction of action potentials in the ureter of rat.
Am. J. Physiol. 181, 651-660
- (26) Sleator, W. and H.R. Butcher 1955 Action potentials and
pressure changes in ureteral peristaltic waves.
Am. J. Physiol. 180, 261-276
- (27) Weidmann, S. 1956 Elektrophysiologie der Herzmuskelfaser.
Huber, Bern
- (28) Woodbury, J.W. 1962 Cellular electrophysiology of the
heart. from the Handbook of Physiology Circulation-1
Chapter II 237-286
- (29) Woodbury, J.W. and Brady, A.J. 1956 Intracellular
recording from moving tissues with a flexibly mounted
ultramicroelectrode. Science, 123 100-101

FIGURES 1 AND 2. NORMAL PATTERNS OF ACTION POTENTIAL IN THE
URETER MUSCLE OF CAT (FIG. 1) AND GUINEA PIG (FIG. 2).
Time interval 200 msec. Voltage calibration 50 mV.
See text in detail.

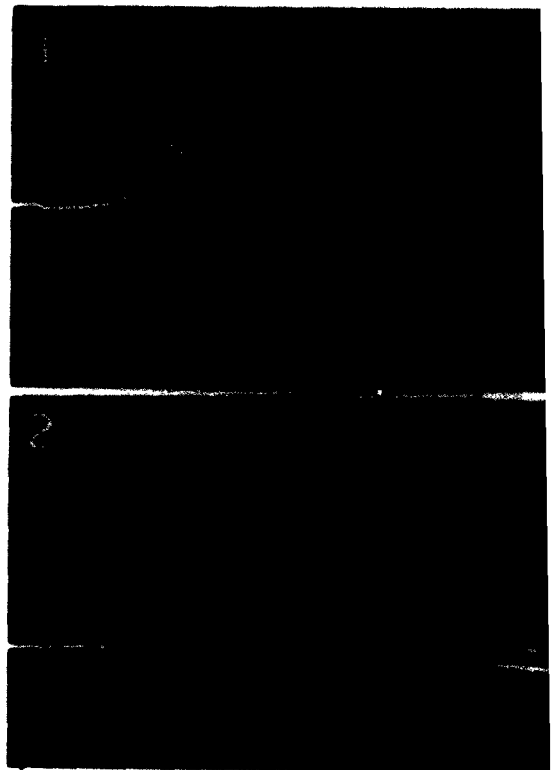
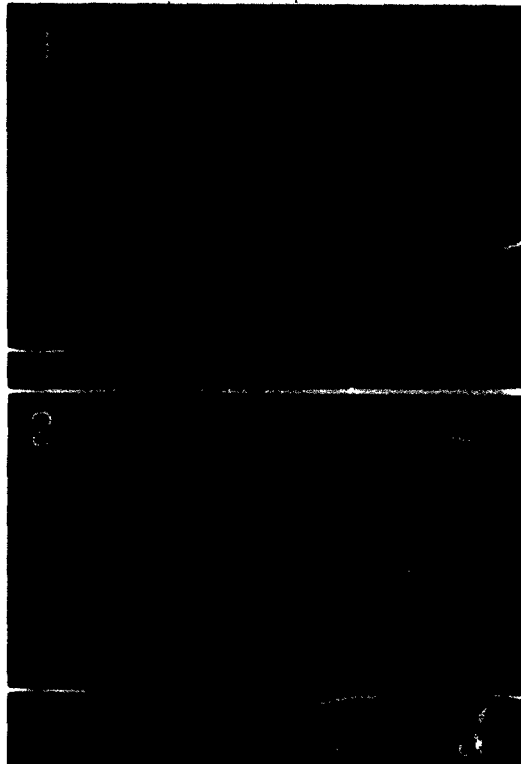


FIGURE 3. ACTION POTENTIALS OF URETER SMOOTH MUSCLE OF CAT.

Stimulus artefact shows the time when the rectangular stimulus of about 50 msec duration was applied. The second stimulus was applied at various time intervals after the first stimulus. Voltage calibration 20 mv. Time:200msec.

FIGURE 4 ACTION POTENTIALS OF URETER MUSCLE OF CAT WHICH WERE ELICITED IN RESPONSE TO THE REPETITIVE STIMULATIONS WITH VARIOUS FREQUENCIES.

Stimulus frequency in 1 is 0.5/sec; in 2, 1/sec; in 3 2/sec. Voltage calibration 20 mv. Time interval 200msec.

FIGURE 5 SUPERIMPOSED ACTION POTENTIALS WHICH WERE SUCCESSIVELY ELICITED BY THE REPETITIVE STIMULATIONS IN CAT URETER MUSCLE.

Tracing 1,2,3 and 4 in each recording (A,B and C) show the potentials produced by the first, second, third and fourth stimuli respectively. In each recording the stimulus artefacts of all the tracings were superimposed. In A, stimulus frequency is 0.5/ sec; in B, 1/sec and in C, 2/ sec.



FIGURE 6. A. ACTION POTENTIALS OF URETER SMOOTH MUSCLE OF GUINEA PIG WHICH WERE ELICITED IN RESPONSE TO THE REPETITIVE STIMULATIONS.

1. control 2. Action potential produced by the stimulus which was applied 3.6 sec after the completion of the repolarization of the first action potential. 3. Action potential by the stimulus of 1.6 seconds after the second action potential. Horizontal bar on the upper right hand of each tracing shows the level of the resting potential.

B. DIAGRAM SHOWING CHANGES IN THE CONDUCTION VELOCITY, THE DURATION AND THE RATE OF RISE OF ACTION POTENTIAL WITH REFERENCE TO THE TIME WHEN STIMULUS WAS APPLIED.

Superimposed tracings of three action potentials of Fig 6 -A are shown in the top. The numerals indicated in the graph show the measured values. Time zero in the abscissa means the time when the repolarization of the previous potential was completed.

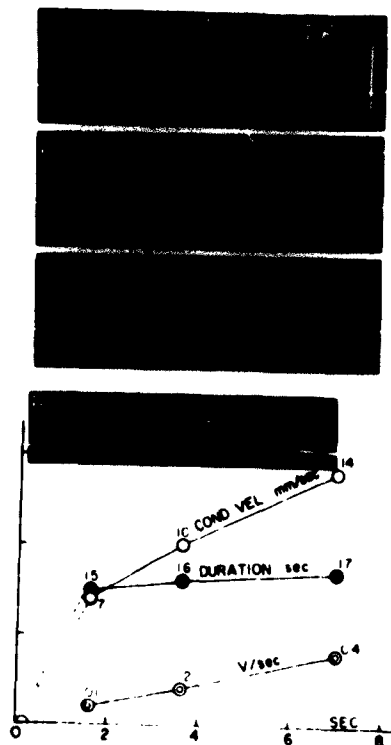
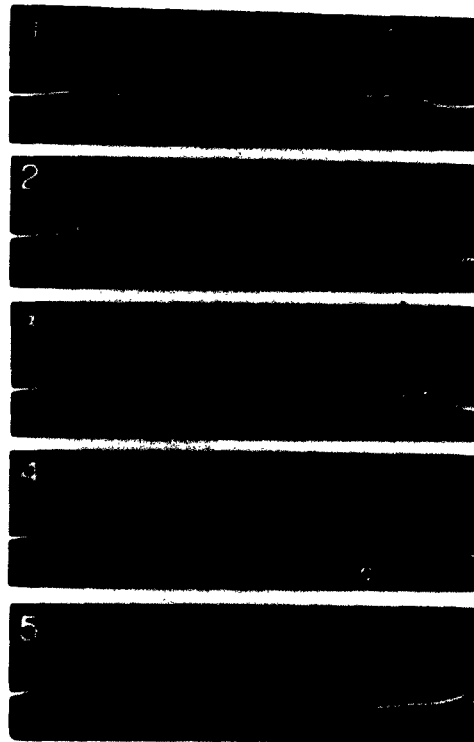


FIGURE 7. SPONTANEOUS SPIKE POTENTIALS AND THE VARIOUS PATTERNS OF ACTION POTENTIALS PRODUCED BY THE STIMULUS IN URETER MUSCLE OF GUINEA PIG.

Rectangular stimulations of about 500 msec duration were applied at various time intervals after the completion of repolarization of spontaneous spike potential. The time intervals in tracings 1-5 were 370, 480, 600, 1480 and 2160 msec, respectively. Voltage calibration 20 mV. The time marks indicated under each tracing show 2 seconds intervals.



FIGURES 8 AND 9. EFFECTS OF TEMPERATURE OF EXTRACELLULAR FLUID ON THE ACTION POTENTIALS OF URETER SMOOTH MUSCLE OF CAT (FIG. 8) AND GUINEA PIG (FIG. 9).

Voltage calibration 50 mV. Time intervals 200 msec. Horizontal bar on the right hand of each tracing in Fig. 9 shows the level of the resting potential.

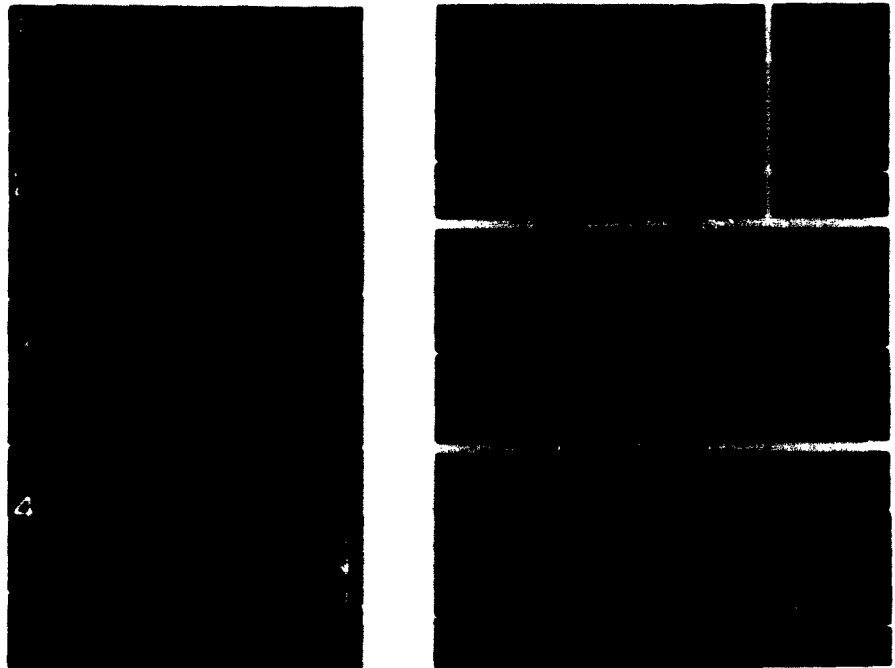


FIGURE 10 CHANGES IN ACTION POTENTIAL PATTERN DUE TO THE REPLACEMENT OF EXTRACELLULAR SODIUM CHLORIDE BY SUCROSE.

- 1 Control in Ringer Krebs's solution
 - 2 50 per cent $[Na^+]$ solution.
 - 3 25 per cent $[Na^+]$ solution after 6 minutes.
 - 4 25 per cent $[Na^+]$ solution after 40 minutes.
- stimulus artefact in each tracing shows the time when the rectangular stimulation of about 50 msec. duration was applied. Time interval 200 msec, voltage calibration 50 mv.

FIGURE 11 CHANGES IN ACTION POTENTIAL PATTERN DUE TO THE REPLACEMENT OF EXTRACELLULAR SODIUM BY CHOLINE CHLORIDE.

- 1 control
 2. 50 per cent sodium solution after 10 minutes.
 3. after 40 minutes.
 4. 25 per cent sodium solution after 10 minutes.
- Stimulus duration 100 msec. Recordings were made approximately 1.0 cm from the stimulating electrode.

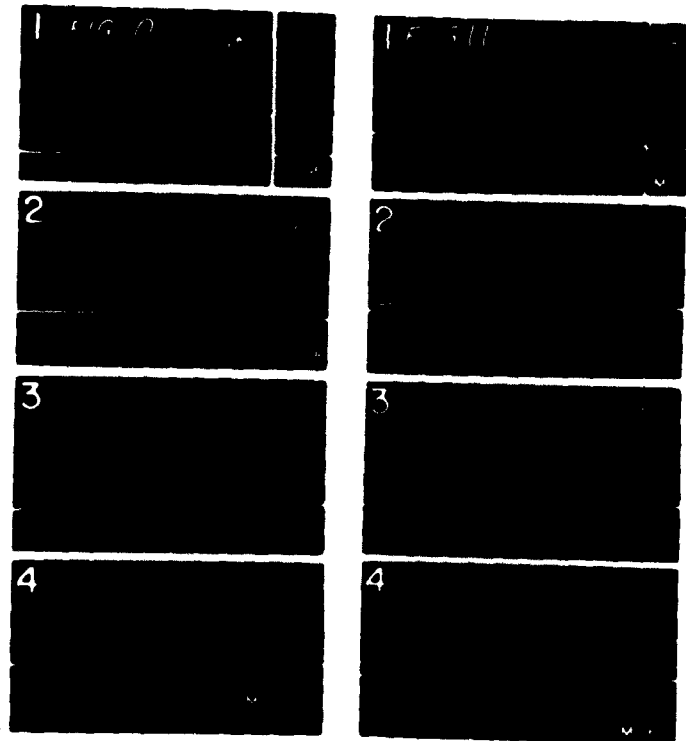


FIGURE 12 SERIES OF RECORDS WHERE EXTRACELLULAR SODIUM CONCENTRATION WAS REDUCED STEPWISE BY THE SUBSTITUTION WITH TRIS CHLORIDE.

1. Control 2. 50 per cent $(Na^+)_o$ solution 3. 25 per cent $(Na^+)_o$ solution 4. 12.5 per cent $(Na^+)_o$ solution 5 and 6 $(Na^+)_o$ -free solution 7. 5 minutes after immersing in normal solution 8. 20 minutes later. Stimulus of 100 msec duration was applied 6 mm from the recording site in tracing 1, 2, 3, and 7: in 4 and 5 4 mm: and 1 mm in 6. In tracing 8 spontaneous action potential is recorded.

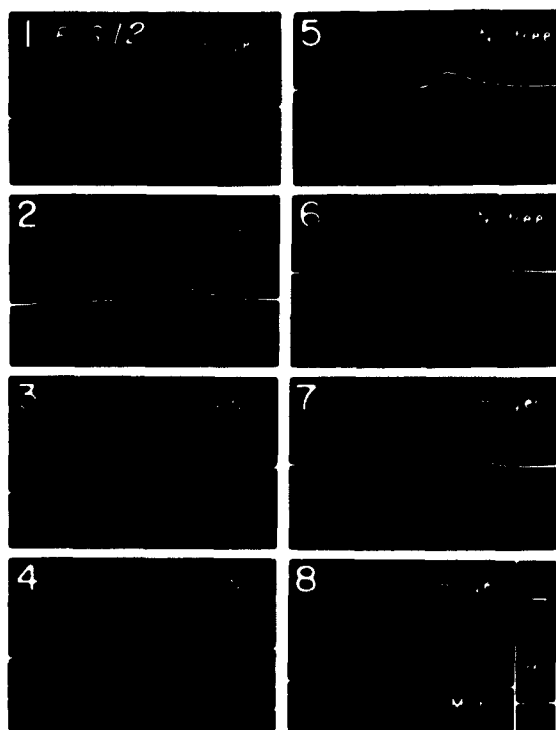


FIGURE 13-15. CHANGES IN THE HEIGHT, THE RISING RATE, AND THE DURATION OF ACTION POTENTIAL BY THE SODIUM DEFICIENCY. The average value from 15 to 30 records were plotted against the concentration of extracellular sodium chloride (per cent normal). Vertical bars show standard deviations.

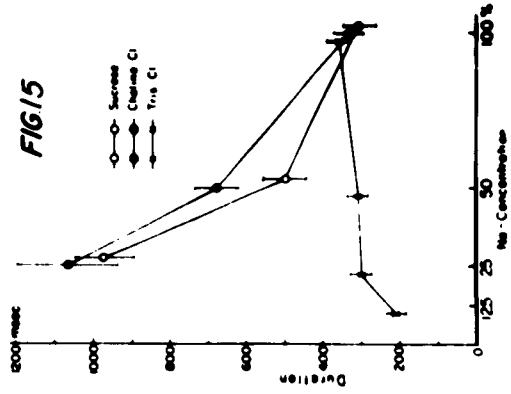
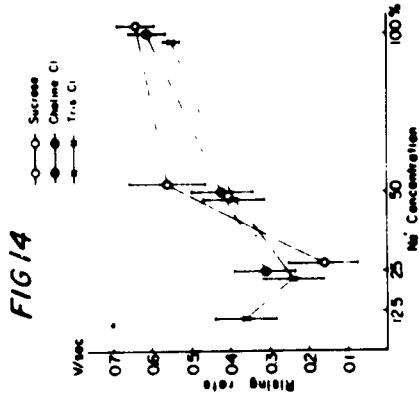
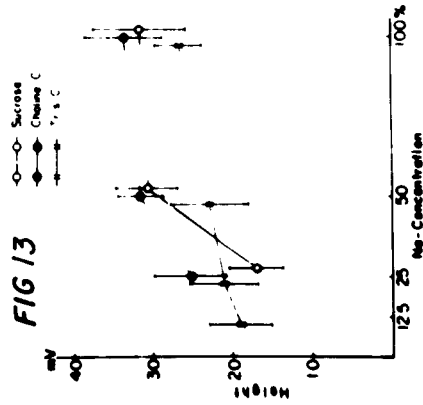


FIGURE 16. CHANGES IN ACTION POTENTIAL PATTERN DUE TO THE REPLACEMENT OF EXTRACELLULAR SODIUM CHLORIDE BY SUCROSE.

1. Control in Ringer-Krebs solution.
2. 50 per cent (Na^+) solution, after 5 minutes.
3. after 20 minutes. 4. 40 minutes later.

Stimulus artefact in each tracing shows the time when the rectangular stimulation of about 50 msec duration was applied. Time interval 200 msec. Voltage calibration 50 mV.

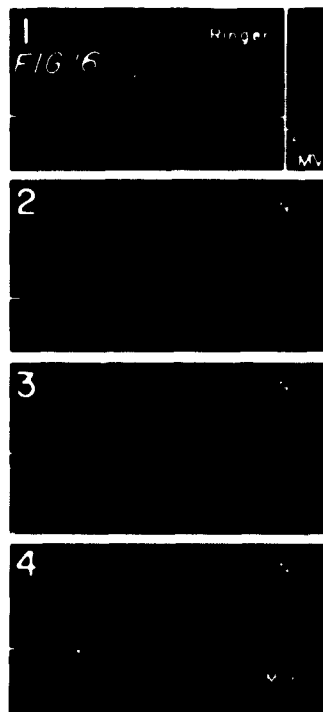


FIGURE 17. SERIES OF RECORDS WHERE EXTRACELLULAR SODIUM CONCENTRATION WAS REDUCED STEPWISE BY THE SUBSTITUTION WITH CHOLINE CHLORIDE.

1. Control in Ringer-Krebs solution.
2. 50 per cent (Na^+) solution, after 5 minutes.
3. 20 minutes later.
4. 25 per cent (Na^+) solution.
- 5 - 7. 12.5 per cent (Na^+) solution, 8. (Na^+)-lack solution. Time interval 200 msec, Voltage calibration 50 mV.

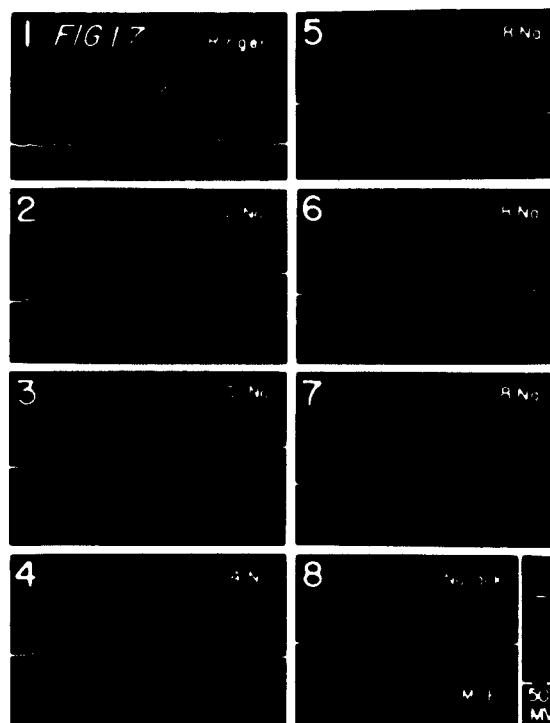


FIGURE 18. CHANGES IN ACTION POTENTIAL PATTERN DUE TO THE REPLACEMENT of extracellular sodium chloride by Tris chloride.

1. Control in Ringer-Krebs solution.
2. 50 per cent $(\text{Na}^+)^0$ solution.
3. 25 per cent $(\text{Na}^+)^0$ solution, 10 minutes later.
4. 20 minutes later. Time interval 200 msec. Voltage calibration 50 mV.

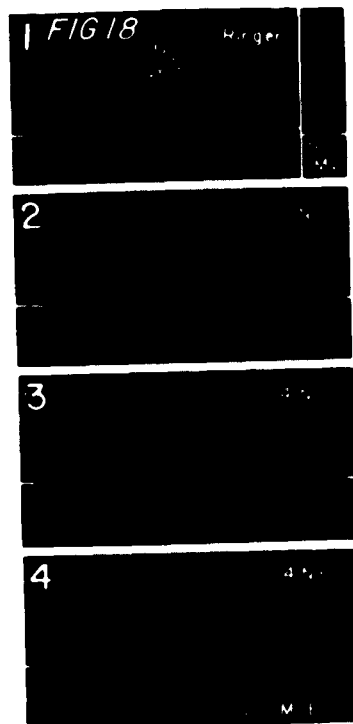


FIGURE 19. EFFECTS OF THE CURRENT INTENSITY ON THE ACTION POTENTIAL OF THE PRETER OF GUINEA PIG.

1. Cathodal polarization. 2. Anodal polarization

An arrow indicates the onset of the current application.

Time interval 2 sec. Voltage calibration 20 mV.

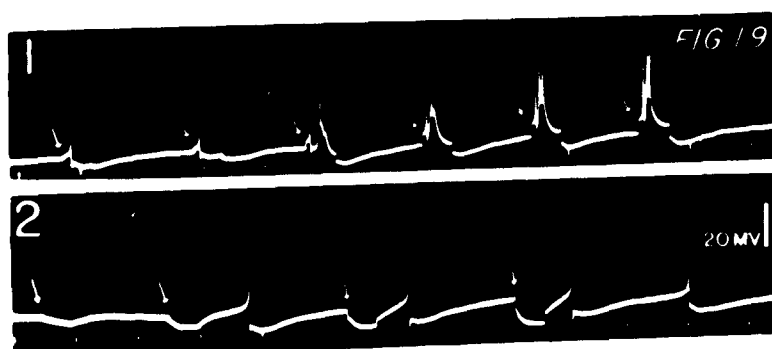


FIGURE 20 ELECTRONMICROGRAPHS OF A LONGITUDINAL SECTION
OF THE SQUILLA MYOCARDIUM.

Mag X 7350 The scale represents one micron.

Thick invaginations of sarcolemmal membrane transverses the myofibril at the Z-line level. This indicates that the extracellular space is directly in contact with the centre of myofilaments, within a very short distance.



FIGURE 21 HIGH MAGNIFICATION OF THE MYOFIBRILS OF STOMATOPOD MYOCARDIUM.

Magnification X 35000

Double myofilament arrangements are illustrated, suggesting the similar structure to that of the vertebrate striated muscles. Thick dots around the myofibrils are glycogen granules. Vesicular structures around the myofibrils are the transverse section of the longitudinal endoplasmic reticulum. A part of mitochondria is illustrated at the bottom of this picture.

